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ORIGINAL REPORT

Development of a Multimarker Assay for Early Detection of Ovarian Cancer

Zoya Yurkovetsky, Steven Skates, Aleksey Lomakin, Brian Nolen, Trenton Pulsipher, Francesmary Modugno, Jeffrey Marks, Andrew Godwin, Elieser Gorelik, Ian Jacobs, Usha Menon, Karen Lu, Donna Badgwell, Robert C. Bast Jr, and Anna E. Lokshin

See accompanying editorial on page 2128

From the University of Pittsburgh Cancer Institute; Departments of Epidemiology, Pathology, Medicine, and Obstetrics/Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh; Fox Chase Cancer Center, Philadelphia, PA; Biostatistics Center, Massachusetts General Hospital; Harvard Medical School, Boston; Department of Physics, Massachusetts Institute of Technology, Cambridge, MA; Department of Surgery, Duke University Medical Center, Durham, NC; Departments of Gynecologic Oncology and Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, TX; and Department of Gynecological Oncology, Institute of Women's Health, University College London, London, United Kingdom.

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Corresponding author: Anna E. Lokshin, MD, University of Pittsburgh Hillman Cancer Center, 5117 Centre Ave, Pittsburgh, PA 15213; e-mail: lokshina@pitt.edu.

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ABSTRACT

Purpose
Early detection of ovarian cancer has great promise to improve clinical outcome.

Patients and Methods
Ninety-six serum biomarkers were analyzed in sera from healthy women and from patients with ovarian cancer, benign pelvic tumors, and breast, colorectal, and lung cancers, using multiplex xMAP bead-based immunoassays. A Metropolis algorithm with Monte Carlo simulation (MMC) was used for analysis of the data.

Results
A training set, including sera from 139 patients with early-stage ovarian cancer, 149 patients with late-stage ovarian cancer, and 1,102 healthy women, was analyzed with MMC algorithm and cross validation to identify an optimal biomarker panel discriminating early-stage cancer from healthy controls. The four-biomarker panel providing the highest diagnostic power of 86% sensitivity (SN) for early-stage and 93% SN for late-stage ovarian cancer at 98% specificity (SP) was comprised of CA-125, HE4, CEA, and VCAM-1. This model was applied to an independent blinded validation set consisting of sera from 44 patients with early-stage ovarian cancer, 124 patients with late-stage ovarian cancer, and 929 healthy women, providing unbiased estimates of 86% SN for stage I and II and 95% SN for stage III and IV disease at 98% SP. This panel was selective for ovarian cancer showing SN of 33% for benign pelvic disease, SN of 6% for breast cancer, SN of 0% for colorectal cancer, and SN of 36% for lung cancer.

Conclusion
A panel of CA-125, HE4, CEA, and VCAM-1, after additional validation, could serve as an initial stage in a screening strategy for epithelial ovarian cancer.

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INTRODUCTION

Ovarian cancer is the fourth most frequent cause of death from cancer in women in Europe and the United States.[1-3] Because ovarian cancers typically cause few specific symptoms, more than 70% of patients are diagnosed with advanced disease, where 5-year survival rates are less than 30%.[1,3] In contrast, the 25% of patients who are diagnosed with stage I disease have a 5-year survival rate of up to 90%, and patients with stage II disease have a 5-year survival rate of up to 70%.[2,3] Therefore, early detection of ovarian cancer has great promise to improve clinical outcome.

At present, no screening techniques are recommended for early detection of ovarian cancer in the general population. CA-125, the most frequently used serum biomarker for ovarian cancer, has a sensitivity (SN) of only 50% to 60% for early-stage disease in postmenopausal women when specificity (SP) is set at 99%.[4-6] Transvaginal sonography (TVS), computed tomography, magnetic resonance imaging, and power Doppler offer less than 90% SN for early ovarian cancer, and their expense and relatively high false-positive rates preclude annual screening.[7-9] Considering the low prevalence of ovarian cancer, a screening strategy must achieve a minimum SP of 99.6% and an SN of more than 75% for early-stage disease to avoid an unacceptable level of false-positive results and achieve a positive predictive value of 10%.[10,11] Using TVS as a second-line test, previous CA-125–based screening studies indicate that a first-line SP of 98% for an annual test could assure required SP ($>$ 99.6%) and positive

Table 1. Characteristics of the Patient Population

Histology	Training Set 2												Validation Set										
	No. of Patients	Race (No. of patients)				Grade (No. of patients)			Age (years)				No. of Patients	Race (No. of patients)				Grade (No. of patients)			Age (years)		
		Asian	Black	Hispanic	White	1	2	3	Range	Median	Mean			Asian	Black	Hispanic	White	1	2	3	Range	Median	Mean
Healthy postmenopausal	1,102	52	87	42	921				48-87	56	57.8		929	42	75	23	789				48-77	55	55.4
Ovarian cancer, postmenopausal, stages IA-IIB	139	5	6	126	2	53	35	51	45-85	59	61.2		44	2	3	1	38	5	10	29	47-87	62	62.6
Endometrioid	42												20										
Mucinous	29												5										
Serous	27												10										
Clear cell	20												8										
Other	21												1										
Ovarian cancer postmenopausal stages IIC-IV	149					42	50	57	48-87	66	65.1		124	3	4	3	95	2	47	75	48-87	64	65.6
Endometrioid													39										
Mucinous													4										
Serous													47										
Clear cell													11										
Other													5										
Benign postmenopausal													296	12	16	9	259				49-85	64	62.7
Breast cancer													210	7	50	6	147				53-82	63	64.4
Lung cancer													74	1	4	2	67				47-91	69	67.8
Colorectal cancer													31	0	2	1	28				49-86	63	64.0

predictive value (> 10%) and would reduce the number of ultrasound examinations performed annually to a cost-effective level of 2%.[10,11]

Similar to CA-125, several other individual ovarian cancer–associated serum protein biomarkers lack sufficient SN or SP for detection of early-stage disease.[12-16] Recently, combinations of serum tumor markers have achieved greater SN than individual markers, while maintaining high SP. Two combinations, CA-125, CA 72-4, CA 15-3, and M-CSF[17] and CA-125, apolipoprotein A1, truncated form of transthyretin, and a cleavage fragment of inter–α-trypsin inhibitor heavy chain H4,[18] substantially improved test accuracy over CA-125 alone, with SNs of 70% to 73% at an SP of 97% to 98%. A panel of six biomarkers (CA-125, leptin, prolactin, IGF-II, MIF, and osteopontin) reportedly exhibited an SN of 95.3% at an SP of 99.4% for patients with all stages of ovarian cancer.[19] However, none of the previous studies have evaluated selectivity of panels for ovarian cancer versus benign disease and other malignancies, where selectivity is 1 – SN of the test when evaluated on benign disease and other malignancies for a given SP in controls. Therefore, the need still exists to develop a diagnostic assay that detects stages I and II ovarian cancer with high SN at 98% SP and high selectivity for ovarian cancer in a larger population of patients with early disease. In the present study, we used a multiplexing approach to analyze 96 candidate serum proteins to identify and validate a biomarker combination with the highest power to detect early-stage ovarian cancer and examine its cancer selectivity.

PATIENTS AND METHODS

Patient Populations

The study population was comprised of 2,031 healthy postmenopausal women; 456 patients with ovarian cancer in stage IA (n = 69), IB to IIB (n = 114), and IIC to IV (n = 273); 296 patients with benign pelvic tumors; and postmenopausal women with breast (n = 210), lung (n = 74), and colorectal (n = 31) cancers (Table 1). Samples were obtained from multiple sources (Table 2) and were annotated with information regarding age, cancer diagnosis, stage, histology, and grade (Table 1). The local institutional review boards approved the protocols for use of each sample collection.

Collection and Storage of Blood Serum

Serum samples were collected before surgery and administration of anesthesia. Procedures for serum collection, processing, and storage have been previously described.[20] Blood processing was similar for all samples collected at the contributing centers.

Table 2. Sources of Serum Samples

Cancer and Menopausal Status	No. of Samples					
	UPCI	GOG	MDACC	FCCC	Duke	UI
Ovarian stage I-IIB, postmenopausal		155	26	2		
Ovarian stage I-II, premenopausal		62				
Ovarian stage IIB-IV, postmenopausal		168	84			21
Benign pelvic, postmenopausal		183	100	13		
Benign pelvic, premenopausal		15				
Breast postmenopausal					210	
Lung postmenopausal	37				37	
Colorectal postmenopausal	31					
Healthy postmenopausal	46	142	1,783	62		
Healthy premenopausal		63				

Abbreviations: UPCI, University of Pittsburgh Cancer Institute; GOG, Gynecologic Oncology Group Blood Bank; MDACC, The University of Texas M. D. Anderson Cancer Center; FCCC, Fox Chase Cancer Center; Duke, Duke University; UI, University of Iowa.

Sources of Bead-Based Immunoassays

The xMAP bead-based technology (Luminex, Austin, TX) permits multiplexed analysis of several analytes in one sample. Ninety-six bead-based xMAP immunoassays for potential ovarian cancer or epithelial cancer serum biomarkers used in this study are listed in Table 3. All assays were research grade. Antibodies for kallikreins were a generous gift of Eleftherios Diamandis, MD (University of Toronto, Toronto, Ontario, Canada). The inter-assay variability of each assay was 1.5% to 6%. The intra-assay variability for assays performed on the same day was 3% to 9%. The intra-assay variability for assays performed at different days was 5% to 20%, depending on whether the same lot of reagents was used.[67-69] Each bead-based assay was validated in comparison with the corresponding enzyme-linked immunosorbent assay based on the same antibody pair and has demonstrated 89% to 98% correlation (data presented on the University of Pittsburgh Web site[70] for in-house assays; performance of purchased assays was in agreement with that claimed by a manufacturer).

Multiplex Analysis

Assays were performed according to manufacturers' protocols as previously described.[21] Samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA). For each analyte, 100 beads were analyzed and means were calculated. Analysis of experimental data was performed using four-parameter logistic curve fitting to the standard analyte curves.

Statistical Analysis

All development of statistical models for distinguishing ovarian cancer patient cases from controls was restricted to the training set until one panel and one model of combining the candidate biomarkers in the panel were selected. A Metropolis algorithm with Monte Carlo simulation (MMC) was used for analysis of the data.[71-73] In MMC analysis, the scoring function (SF) for a specific biomarker panel was constructed as a linear combination of logarithms of biomarker concentrations (with minimal detectable concentration added to concentration to reduce the effect of large relative errors near zero concentrations). The Monte Carlo optimization was then used to determine the coefficients in this linear combination that, for the training set, provide the best SN at the desired SP. The cutoff was adjusted at the each iteration of parameter estimates to maintain the desired SP. When a range of cutoffs gave the same SP, we used the average cutoff. The advantage of using the number of misdiagnosed cancers as the optimization criterion is that it does not rely on any assumptions regarding the statistical distribution of the data, and as a result, the optimal linear combination is practically insensitive to the presence of outliers and erroneous data. However, the choice of SF does restrict the combination of biomarkers to linear combinations to provide the optimum separation. Therefore, no patient cases were excluded from this analysis. All possible panels consisting of two, three, and four biomarkers were evaluated for SN at 95% SP in the preliminary training set. For each panel size, the 500 panels with the best SN at 95% SP on the full data set were re-estimated with cross validation. For cross validation, 20% of participants were randomly excluded from the data set, and the rest were used as at training set to build the optimal SF. The resultant model was applied to the excluded participants, and this process was repeated 400 times. A smaller subset of biomarkers that comprised the top 20 panels was then re-evaluated in a larger training set at 98% SP. One optimal panel was chosen that offered high cross-validated SN for both early- and late-stage ovarian cancer at 98% SP. This sole panel and method of combination was evaluated in the validation set.

Univariate comparisons were made using the Wilcoxon rank sum test or *t* test for log-transformed data. Exact CIs for binomial proportions are provided by the R function binom.test, which inverts the exact binomial test. Comparisons between different samples sources were performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test.

Table 3. Multiplexed Biomarkers

Biologic Group and Source*	Proteins	Plex No.†
Cytokines/chemokines		
Invitrogen/Biosource	Eotaxin-1, TNFR1, TNFR2, IL-1Rα, IL-2R, IL-6R	1
Millipore/Linco	IL-1b, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-13, G-CSF, GM-CSF, IFNγ, TNF-α, IP-10, MCP-1, MIP-1α, MIP-1β, MIF, CD40L, fractalkine[21,22]	2
Growth/angiogenic/factors		
Invitrogen/Biosource	EGF,[21] VEGF, bFGF, HGF, NGF,[23] EGFR[13,24]	1
Luminex Core Facility, University of Pittsburgh	Her2/neu,[25,26] IGFBP-1[27]	3
Antiangiogenic factors		
Luminex Core Facility, University of Pittsburgh	Angiostatin, endostatin, thrombospondin[28,29]	4
Cancer antigens		
Luminex Core Facility, University of Pittsburgh	CA-125,[30,31] CA 15-3,[32,33] CEA[34-36]	5
Luminex Core Facility, University of Pittsburgh	CA 19-9,[37] CA 72-4,37,38 AFP,[39] SCC[40]	6
Apoptotic proteins		
Luminex Core Facility, University of Pittsburgh	Cyfra 21-1[15,41]	5
Invitrogen/Biosource	DR5[42]	1
Millipore/Linco	Fas, FasL[43]	7
Proteases/binding proteins		
Luminex Core Facility, University of Pittsburgh	Kallikreins 8 and 10[44,45]	5, 6
R&D Systems	MMP-1 to MMP-13[46-48]	8
R&D Systems	TIMP-1 to TIMP-4	9
Adhesion molecules		
Millipore/Linco	ICAM, VCAM, E-selectin[49]	10
Hormones		
Millipore/Linco	Prolactin, TSH, LH, ACTH, GH[50,51]	11
Luminex Core Facility, University of Pittsburgh	βHCG[52,53]	6
Adipokines		
Millipore/Linco	Adiponectin, leptin, resistin[54-57]	12
Other markers		
Luminex Core Facility, University of Pittsburgh	Mesothelin[58]	6
Luminex Core Facility, University of Pittsburgh	HE4[59]	13
Millipore/Linco	tPAI-1, active PAI-1,[60] MPO[61]	14
Millipore/Linco	Apolipoproteins (Apo) A1, AI, AII, B, CII, CIII, E[18,62,63]	15
Luminex Core Facility, University of Pittsburgh	TTR[18]	16
Millipore/Linco	Insulin,[64] osteopontin,[56] osteoprotegerin,[65] osteocalcin[66]	17

*Invitrogen/Biosource, Camarillo, CA; Millipore/Linco, St Louis, MO; Luminex Core Facility, University of Pittsburgh, Pittsburgh, PA; and R&D Systems, Minneapolis, MN.
†Plex No. indicates multiplexed panel (i.e., biomarkers that were analyzed simultaneously).

RESULTS

Bead-Based Immunoassay Analysis of Biomarker Serum Concentrations in Patients With Ovarian Cancer

Because of the low prevalence of ovarian cancer, the required minimum SP of serum test should be 98% when combined with TVS

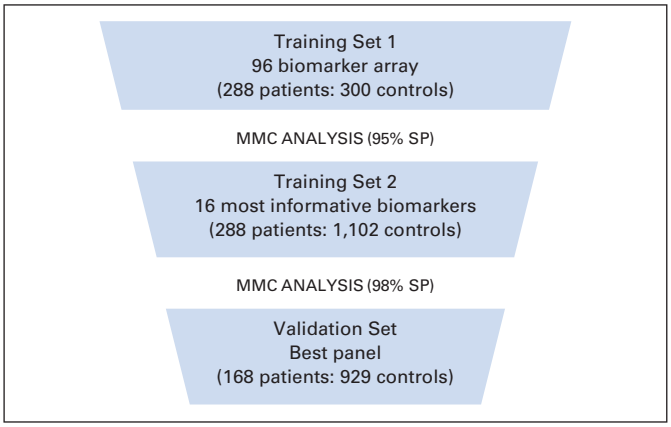

Fig 1. Flowchart of experimental design. MMC, Metropolis algorithm with Monte Carlo simulation; SP, specificity.

as a second-line test.[10,11] Accurate assessment of such high SP requires at least 1,000 healthy participants. Therefore, our strategy was to first analyze the preliminary smaller training set using MMC algorithm to select a subset of biomarker combinations (panels) providing the highest SN at 95% SP and then to use an expanded training set containing more than 1,000 healthy sera to select and validate a panel offering the highest SN at 98% SP (Fig 1).

Serum samples from the preliminary training set of postmenopausal women consisting of 48 patients with stage IA, 91 patients with stage IB to IIB, and 149 patients with stage III to IV ovarian cancer and 276 age-matched healthy controls were analyzed using multiplexed bead-based immunoassays for 96 cancer-associated biomarkers representing proteins with different biologic functions (Table 3). These candidate biomarkers were selected based on the published evidence documenting an association with epithelial cancer development and progression.[13,15,18,21-63,74] Serum concentrations of 35 proteins demonstrated highly significant ($P < .001$) differences between ovarian cancer samples and samples from healthy controls (Data Supplement Fig 1). Circulating concentrations of CA-125, HE4, prolactin, IL-2R, CA 72-4, CA 15-3, CA 19-9, MIF, Cyfra 21-1, TNFR1, TNFR2, IL-7, IL-10, IGFBP1, IL-6, TNF-α, GH, TSH, ACTH, TIMP-1, and osteopontin were significantly ($P < .001$) higher in serum of patients with early-stage ovarian cancer compared with healthy women, whereas concentrations of eotaxin-1, MMP-2, MMP-3, VCAM-1, EGFR, ErbB2, FSH, LH, CD40L, ApoA1, adiponectin, TTR, and osteocalcin were significantly ($P < .001$) lower in serum of patients with early-stage ovarian cancer compared with healthy women. Serum levels of HE4, prolactin, IL-2R, CA 72-4, CA 15-3, CA 19-9, Cyfra 21-1, TNFR1, TNFR2, IL-7, IL-10, IGFBP1, IL-6, TNF-α, TSH, MMP-7, eotaxin-1, VCAM-1, ErbB2, FSH, LH, ApoA1, adiponectin, TTR, and CD40L differed significantly ($P < .01$) between patients with early-stage (stages I and II) and late-stage (stages III and IV) ovarian cancer (Data Supplement Fig 1). Circulating concentrations of CA-125, HE4, IL-2R, CA 72-4, CA 15-3, CA 19-9, Cyfra 21-1, TNFR1, TNFR2, IL-7, IL-10, IGFBP1, IL-6, TIMP-1, osteopontin, MMP-7, MMP-2, EGFR, FSH, LH, ApoA1, and TTR were significantly ($P < .05$) different between early ovarian cancer and benign pelvic disease. No significant differences in serum concentrations of any of the biomarkers between stages I and II were found (data not shown).

Statistical Analysis of Multimarker Panels

MMC algorithm was applied to the preliminary training set consisting of sera from patients with ovarian cancer (stage IA, n = 54; stage IB to II, n = 85; and stage IIC to IV, n = 149) and 276 healthy women. Performance of two-, three-, four-, and five-biomarker panels was compared. Four-biomarker panels offered superior performance compared with two- and three-biomarkers panels, whereas using five-biomarker panels did not result in significant improvement. Thirty-five four-biomarker panels with statistically indistinguishable classification accuracy for early-stage disease were identified (Table 4). These panels represented various combinations of the following 16 biomarkers: eotaxin-1, IL-2R, MMP-2, MMP-3, Cyfra 21-1, ErbB2, EGFR, CEA, CA72-4, IGFBP-1, VCAM-1, FSH, GH, HE4, MMP-7, and CA-125. Distributions of 16 biomarkers in samples obtained from different cancer centers were analyzed by one-way ANOVA with Tukey's multiple comparison test to assess bias between the centers (Data Supplement Fig 2). For VCAM-1, eotaxin-1, Cyfra 21-1, ErbB2, EGFR, MMP-3, IGFBP-1, IL-2R, and GH, the differences

Table 4. Sensitivities of Different Panels After Cross Validation at 95% Specificity Using the MMC Algorithm

Marker				Sensitivity (%)
M1	M2	M3	M4	
CA-125	Eotaxin-1	CEA	HE4	96.1
CA-125	CEA	VCAM-1	HE4	95.7
CA-125	Eotaxin-1	EGFR	HE4	94.6
CA-125	Eotaxin-1	IGFBP-1	HE4	94.7
CA-125	Eotaxin-1	HE4	MMP-7	94.2
CA-125	VCAM-1	GH	MMP-7	93.6
CA-125	CA 72-4	VCAM-1	HE4	94.2
CA-125	CEA	IGFBP-1	HE4	94.1
CA-125	Eotaxin-1	ErbB2	HE4	94.3
CA-125	IGFBP-1	VCAM-1	MMP-7	94
CA-125	IGFBP-1	VCAM-1	HE4	94.5
CA-125	Eotaxin-1	VCAM-1	HE4	94.6
CA-125	MMP-3	VCAM-1	MMP-7	93.5
CA-125	EGFR	CEA	HE4	93.7
CA-125	CEA	IL-2R	HE4	94.1
CA-125	Eotaxin-1	IL-2R	HE4	94
CA-125	Eotaxin-1	GH	HE4	94.3
CA-125	VCAM-1	GH	HE4	93.9
CA-125	ErbB2	CEA	HE4	93.6
CA-125	Eotaxin-1	CA 72-4	HE4	94.1
CA-125	Eotaxin-1	IGFBP-1	VCAM-1	93.6
CA-125	Eotaxin-1	CEA	IGFBP-1	93.2
CA-125	Eotaxin-1	MMP-2	HE4	94
CA-125	MMP-2	CEA	HE4	93.9
CA-125	Eotaxin-1	VCAM-1	MMP-7	93.3
CA-125	CEA	CA 72-4	HE4	93.3
CA-125	EGFR	VCAM-1	HE4	94
CA-125	MMP-3	CEA	HE4	93.3
CA-125	Cyfra 21-1	CEA	HE4	93
CA-125	Eotaxin-1	MMP-3	MMP-7	93.2
CA-125	Eotaxin-1	IGFBP-1	GH	92.6
CA-125	MMP-2	VCAM-1	MMP-7	93.2
CA-125	Eotaxin-1	IGFBP-1	MMP-7	93.3
CA-125	Eotaxin-1	MMP-2	IGFBP-1	93
CA-125	Eotaxin-1	IL-2R	HE4	94.1

Abbreviation: MMC, Metropolis algorithm with Monte Carlo simulation.

between sites were not significant, whereas for CA-125, HE4, CEA, MMP-7, FSH, and CA 72-4, statistical significance between samples sources was observed. Even when the differences between sites using values from control participants were statistically significant, the magnitude of the biases was small compared with the differences between cancer patients and controls. For example, for CA-125, the ANOVA was significant, but the point estimates for the differences between sites ranged from 3% to 25%. In comparison, the differences between the cancer patients and controls ranged from 88% to 93%, far larger than the differences between sites.

These biomarkers were reanalyzed in an expanded training set containing 826 additional healthy controls (total N = 1,102) to ascertain performance at 98% SP. A panel selected for best performance was CA-125, HE4, CEA, and VCAM-1; this panel provided 86% SN (95% CI, 79% to 91%) for early-stage ovarian cancer and 93% SN (95% CI, 88% to 97%) for late-stage ovarian cancer at 98% SP (Fig 2). The coefficients of the logarithm of the biomarker concentrations were as follows: CA-125, 0.86; HE4, 0.18; CEA, −0.15; and VCAM-1, −1.44. For comparison, the SN of CA-125 alone at 98% SP was 61% (95% CI, 53% to 69%) for early-stage disease and 83% (95% CI, 76% to 89%) for late-stage disease (Fig 3). The four-biomarker panel offered comparable classification for the four most prevalent histologic types of ovarian cancer—serous (SN = 89%), mucinous (SN = 85%), endometrioid (SN = 90%), and clear cell (SN = 85%) carcinoma. Nonepithelial histologies were classified with an SN of 79%.

Next, the classification results were validated in an independent blinded validation set consisting of 929 healthy controls, 296 benign participants, and 168 patients with ovarian cancer (44 patients with stage I to IIB ovarian cancer, including 14 patients with stage IA and 124 patients with stages IIC to IV ovarian cancer; Table 1). The blinding of validation set was performed by the personnel at the sites providing the biospecimens, and the patient case/control status was unblinded only after the algorithm and its predictions for the validation set were provided back to the sites. The optimal four-biomarker panel classified early-stage cancers with 86% SN (95% CI, 73% to 95%) and late-stage cancers with 95% SN (95% CI, 90% to 98%) at 98% SP, which was significantly higher than CA-125 alone. Of 14 patients with stage IA disease, three were misclassified, resulting in 79% SN (95% CI, 49% to 95%). The four-biomarker set performed well for classification of patients with stage III to IV disease, offering 94% SN (95% CI, 79% to 98%) at 98% SP. Figure 2B demonstrates distributions of scores obtained for analysis of different cancer groups and healthy controls in both training and validation sets by MMC algorithm. This multimarker panel correctly diagnosed 67% (95% CI, 61% to 72%) of 296 blinded benign samples as noncancers.

Selectivity of the Panel for Ovarian Cancer versus Other Epithelial Cancers

The panel of CA-125, HE4, CEA, and VCAM-1 was used to classify a blinded mixed set of patient cases with three other common female cancers, breast (n = 210), colorectal (n = 31), and lung (n = 74). This panel correctly identified 94% (95% CI, 90% to 97%) of breast cancers, 100% (95% CI, 89% to 100%) of colorectal cancers, and 64% (95% CI, 52% to 74%) of lung cancers as non-ovarian cancer.

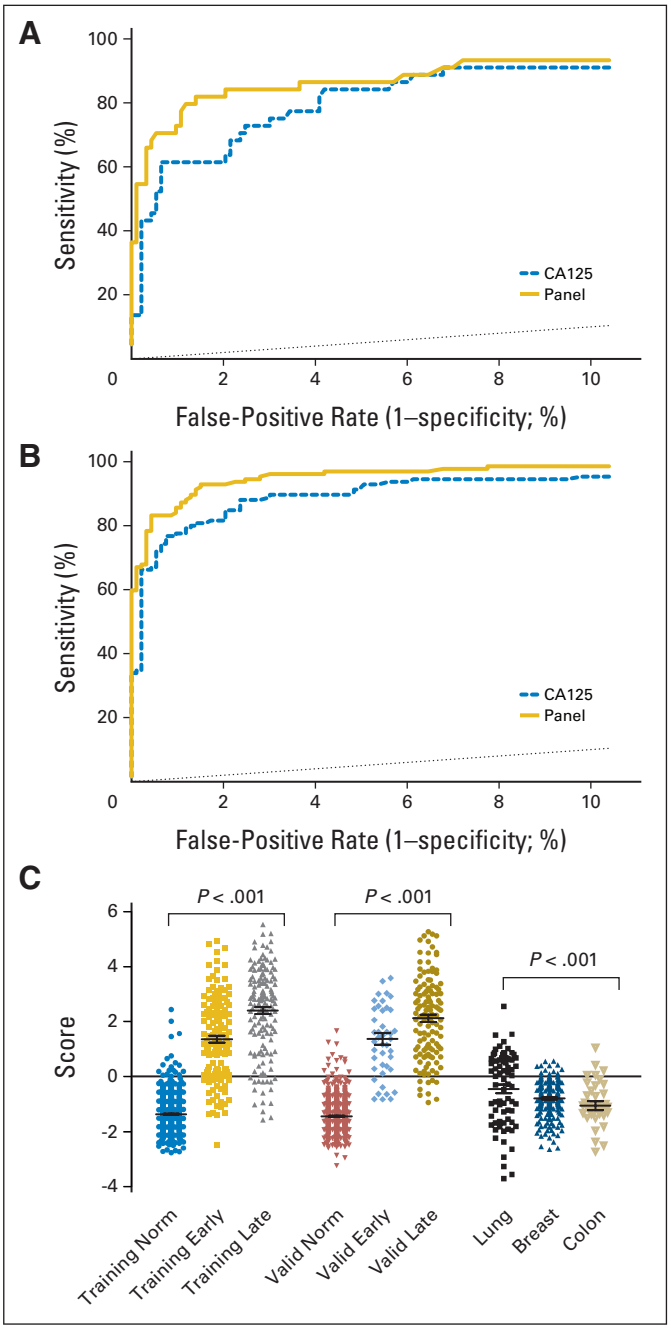

Fig 2. Cumulative receiver operating characteristic (ROC) curves in the training set and distribution of scores in training and validation sets using the four-biomarker panel with Metropolis algorithm with Monte Carlo simulation (MMC): (A) healthy controls versus stage I to IIB ovarian cancer; (B) healthy controls versus stages IIC to IV ovarian cancer. Solid line indicates four-biomarker panel; dashed line indicates CA125. (C) Distributions of scores created by MMC algorithm. *P* value over a group denotes statistical significance of differences between each group member and appropriate healthy control. Breast, colon, and lung cancers are compared with early-stage ovarian cancer in the validation set.

DISCUSSION

A multimarker approach seems to hold substantial promise for detection of early-stage ovarian cancers.[56] The goal of the present

study was to select a panel of biomarkers that would provide high SN and SP for distinguishing early-stage ovarian cancer from healthy controls from a large array of serum proteins with different biologic functions representing various aspects of systemic response to a growing tumor.[12,21,60,75-88] Availability of a bead-based xMAP multiplexing technology was essential for such analysis because sufficient sera would not be available to perform such a large number of conventional enzyme-linked immunosorbent assays.

After performing a multimarker bead-based immunoassay screening, we observed, in agreement with published evidence, elevated serum levels of IGFBP-1, MIF, HE4, IL-2R, CA19-9, CA-125, Cyfra 21-1, CA 72-4, and prolactin and lower levels of FSH, LH, EGFR, TTR, and ApoA1 in patients with ovarian cancer compared with healthy women.[9,37,41,86-89] In addition, to the best of our knowledge, we are the first to report significant elevation of serum levels of GH and ACTH and lower concentrations of eotaxin-1, immunodetectable MMP-2 and MMP-3, and VCAM-1 in serum of patients with ovarian cancer. The mechanisms behind lower MMP-2, MMP-3, and VCAM-1 levels in patients with ovarian and other cancers have yet to be determined. We have discussed biologic functions of these differentially expressed proteins and their putative roles in pathways associated with ovarian carcinogenesis in our previous report.[90]

Using an MMC algorithm, we have identified a combination of four serum proteins, CA-125, HE4, CEA, and VCAM-1, that offered 86% SN at 98% SP for early-stage ovarian cancer in the solely evaluated, independent, blinded validation set. Analysis of TTR and ApoA1 on the basis of the discovery by Zhang et al[18] was performed. Using our antibody combinations, neither of these two biomarkers offered additional information compared with the multimarker panels presented in Appendix Table A1 (online only).

The high SN of 86% achieved with our four-marker panel for distinguishing women with early-stage ovarian cancer from healthy individuals at 98% SP surpasses the SNs previously reported (70% to 73%).[9,37,41,86-89] The fact that the multimarker assay offered high accuracy in a heterogeneous validation set that contained different histologies indicates the likely general utility of the assay for the most common histotypes of ovarian cancer. Of note, in agreement with published evidence, we observed a lower incidence of serous histology in early-stage patients.[91] The panel of biomarkers correctly classified 67% of benign lesions as noncancer. The incidence of benign disease is approximately 2.4 benign cases per each ovarian cancer case.[92] With 67% of benign lesions recognized as noncancer by the four-biomarker panel, only 0.7 benign lesions will be diagnosed as cancer for each true ovarian cancer. SP would be further improved by the subsequent use of TVS to distinguish malignant from benign pelvic lesions in 2% of women classified as at risk for ovarian cancer, further reducing the number of laparotomies for benign disease.[93]

The three most commonly diagnosed cancers in women are breast, lung, and colorectal.[94] The current panel was highly selective for ovarian cancer when compared with breast cancer and moderately selective when compared with lung cancer, suggesting that multimarker assays can be cancer specific. Detection of occasional breast and lung cancers might be facilitated by incorporating mammography and spiral chest computed tomography in the second phase of screening for women with a negative TVS.

The performance of this multimarker panel for detecting ovarian cancer in asymptomatic patients remains to be further determined in longitudinal retrospective studies using the serum banks established by major ongoing prospective trials (eg, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; Women's Health Initiative; United Kingdom Collaborative Trial of Ovarian Cancer Screening). In these studies, the CA-125, HE4, CEA, and VCAM-1 panel may be further modified by the addition of other biomarkers to achieve optimal lead time before clinical diagnosis and high SN and SP. SN of the panel for ovarian cancer could be further improved by incorporating longitudinal information similar to the method developed for CA-125.[95]

In summary, data obtained in this study suggest that a four-biomarker panel may detect 86% of patients with early-stage ovarian cancer with an SP of 98%. This panel could be used as the first-line test in a two-step strategy for early detection, similar to the approach used in the current United Kingdom Collaborative Trial of Ovarian Cancer Screening where increasing CA-125 triggers TVS.[30,96]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Steven Skates, Elieser Gorelik, Robert C. Bast Jr, Anna E. Lokshin

Provision of study materials or patients: Francesmary Modugno, Jeffrey Marks, Andrew Godwin, Karen Lu, Robert C. Bast Jr, Anna E. Lokshin

Collection and assembly of data: Zoya Yurkovetsky, Robert C. Bast Jr, Anna E. Lokshin

Data analysis and interpretation: Zoya Yurkovetsky, Steven Skates, Aleksey Lomakin, Brian Nolen, Trenton Pulsipher, Elieser Gorelik, Usha Menon, Donna Badgwell, Robert C. Bast Jr, Anna E. Lokshin

Manuscript writing: Zoya Yurkovetsky, Steven Skates, Aleksey Lomakin, Brian Nolen, Elieser Gorelik, Ian Jacobs, Usha Menon, Robert C. Bast Jr, Anna E. Lokshin

Final approval of manuscript: Zoya Yurkovetsky, Steven Skates, Aleksey Lomakin, Brian Nolen, Elieser Gorelik, Ian Jacobs, Usha Menon, Donna Badgwell, Robert C. Bast Jr, Anna E. Lokshin

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